

## Diversity of isozyme electrophoretic patterns in *Salvadora oleoides*

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### ABSTRACT

Enzyme electrophoresis can directly reveal genetic polymorphism through demonstrating the multiple forms of a specific enzyme. Isozymic electrophoretic patterns of seven enzymes –Esterase (EST), Acid phosphatase (ACPH), Catalase (CAT), Peroxidase (PRX), Malate dehydrogenase (MDH), Phosphoglucoisomerase (PGI), and Phosphoglucomutase (PGM) were analyzed in *Salvadora oleoides* by native polyacrylamide gel electrophoresis. Data from the migration of the staining zones indicated that five loci control EST, PRX, MDH, while two loci control CAT, PGI, PGM and ACPH is controlled by one locus. These seven enzymes led to resolution of 22 genetic loci. Of these 22 genetic loci, 18 loci were polymorphic, while 4 loci showed monomorphism. Besides showing the loci, isozymic banding pattern of heterozygous genotypes also revealed subunit structure of the enzyme (occurrence of a hybrid band indicated dimeric structure while absence of hybrid band revealed monomeric enzyme).

**Key words:** Isozymes, electrophoresis, allelic variation, *Salvadora oleoides*

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### INTRODUCTION

*Salvadora oleoides* is a small bushy evergreen tree found in India, Pakistan and southern Iran. This tree species is of multipurpose use because of its oil yielding potential, pharmaceutical application, fodder and fuel values and many others. It is suitable for growing in shelterbelts and as windbreaks in desert tracks. The whole plant is used as diuretic, cooling herb, anti-inflammatory agent, wound healing herb and nerve tonic [1]. Its seeds contain 40-50 % of a greenish-yellow fat containing large amounts of lauric and myristic acids, so can be used for making soap, detergent, cosmetics, paints, varnish, lubricants and candles [2]. The seed fat is used in the treatment of rheumatic pains, in preparation of suppositories and as a base for ointments. Fruits are used in the treatment of enlarged spleen, rheumatism and fever [3]. Leaves are used to relieve cough and for treatment of enlarged spleen and fever. This plant holds strong antifungal [4], anti-parasitic, antiviral [5] and antibacterial [6] properties. The young branches and leaves are also favorite fodder for camels because of the high water content (15-36%). Leaves and stem of this plant have shown significant hypoglycemic and hypolipidemic properties and is effective in rheumatic pains [7]. In view of multifaceted utilization, *S. oleoides* has been included in restoration programs of many developing countries in Africa and Asia [8].

Among the major applications of isozymes, they are indicated as the most frequently applied techniques to plant genetic resources management. The ability to observe allelic variation at isozyme loci revolutionized the research in the field of biochemical genetics, population genetics and evolution [9-10]. Isozyme provides an efficient and effective measure of genetic diversity because of their variability, codominant expression and absence of epistasis. In recent times use of molecular marker for study of genetic diversity is increasing [11-12], still isozymes are widely

used because of their relative simplicity and cost effectiveness as compared to molecular markers, particularly in studies of intra and inter-specific variation [13-14]. In the last ten years there has been a major decline in the populations of this plant and area under this genus is diminishing very rapidly thus causing major threat to forest ecosystem. There has been not any study to find isozymic variation in this plant to estimate the range and quantum of existing natural variation which is essential for framing meaningful genetic improvement programme, aimed at sustainable utilization. This paper is the first attempt to study allelic variation at isozymic loci in *S. oleoides*.

#### MATERIALS AND METHODS

The study was performed on *S. oleoides* collected from eleven different locations of Haryana. A total of 500 plants of *S. oleoides* were studied. Leaves from these plants were collected for electrophoretic analysis. Electrophoresis was done on vertical 1.5% polyarylamide gels with a tris glycine, pH 8.3 buffer system. Enzymes extracts were prepared by crushing leaf tissue in liquid nitrogen with extraction buffer [15]. The following seven enzymes were assayed as per standard methods [16-17] with some modifications to obtain better resolution– Esterase (Est-1, Est-2, Est-3, Est-4 Est-5), Acid phosphatase (AcpH-1), Catalase (Cat-1, Cat-2), Peroxidase (Prx-1, Prx-2, Prx-3, Prx-4, Prx-5), Malate dehydrogenase (Mdh-1, Mdh-2, Mdh-3, Mdh-4, Mdh-5), Phosphoglucoisomerase (Pgi-1, Pgi-2) and Phosphoglucomutase (Pgm-1, Pgm-2) (with the loci indicated in the parentheses).

#### RESULTS AND DISCUSSION

Eleven populations (500 plants) along the whole range of *S. oleoides* were analyzed by isozyme electrophoresis that led to resolution of 22 genetic loci. Of these 22 genetic loci, 18 loci were polymorphic, while 4 loci showed monomorphism.

**Esterase (EST):** Five polymorphic zones of activity considered as products of five loci, ranging from Est-1, from most cathodal zone to Est-5 most anodal zone had been observed (Figure 1(A) & (B)). Zone Est-I and Est-4 were represented by segregating single banded (fast and slow) and double banded patterns while the other three zones (Est-2, 3 and 5) had revealed occurrence of two banded (fast or slow) and three banded variants. The segregating patterns of esterase bands at different zones were independent of each other. The single banded and double-banded patterns represented homozygous and heterozygous genotypes respectively and thus revealed monomeric nature of esterase at these two loci. The two banded and three banded patterns at Est-2, Est-3 and Est-5 locus represented homozygous and heterozygous individuals respectively. The present observation could be explained on the basis that more than one electromorphs (conformational isozymes) might arise due to posttranslational (epigenetic causes) differential binding of conjugates (subunits). In these cases the core enzyme remains the same but two polypeptides differed in the differential binding of subunits. At these three loci, the esterase isozymes were dimeric but due to posttranslational modification it showed two banded and three banded patterns in the homozygotes and heterozygotes respectively [18].

**Acid phosphatase (ACPH):** Gel slices stained for acid phosphatase had revealed single zone of activity (Figure 1 (C) & (D)). This zone was represented by three banded and five banded patterns, which might represent homozygotes and heterozygotes respectively.

The present observation in *S. oleoides* did not agree with genetically controlled dimeric (single and triple banded patterns) or tetrameric (single and five banded patterns) enzymes coded by a single locus [19]. Furthermore, the observed ACPH patterns could not be equated with the three banded and five banded patterns (conformational isozymes) of some NAD dependent gene enzyme systems such as alcohol dehydrogenase (ADH) and cytoplasmic malate dehydrogenase (MDH) as the later enzyme system involve secondary modification of primary gene product through differential binding of coenzymes (NAD) [20]. The observed ACPH phenotypes could be explained if we assume that three banded homozygous genotypes has resulted from duplicate alleles (tandem gene duplication). The occurrence of two such duplicate allelic variants at the ACPH locus could adequately explain the two types of observed triple-banded patterns (fast or slow) in *S. oleoides*. ACPH being a dimeric enzyme each of triple-banded pattern in homozygote consisted of two homodimeric end bands and an intermediate heterodimeric band.

The observed ACPH phenotypes could result if in the duplicate alleles, one copy of ACPH gene codes for an enzyme of fast mobility and is linked to a copy of the gene that codes for enzyme of slow mobility. The phenotypes/genotypes of two duplicate allelic variants in *S. oleoides* might be designated as Fast = 1F 2F/1F 2F and

Slow = 1S 2S / 1S 2S of three banded homozygotes. Likewise, the five banded heterozygotes could be referred phenotypically/genotypically as FS=1F 2F/1S 2S. The presumed duplicate alleles at ACPH locus could explain the occurrence of genetically coded three or five-banded ACPH pattern and reveal that the subunit structure of ACPH isozyme is dimeric in *S. oleoides*. Nevertheless, the dimeric nature of this enzyme has been demonstrated in other perennial species [21].

**Catalase (CAT):** Two zones of activity were detected on gels stained for this enzyme system representing two polymorphic loci; Cat-1 and Cat-2 (Figure 2 (G) & (H)). Locus-1 consisted of single banded (fast or slow) and two banded patterns representing homozygous (FF, SS) and heterozygous (FS) genotypes respectively. The occurrence of double banded heterozygotes and single banded homozygotes revealed monomeric enzyme at this locus.

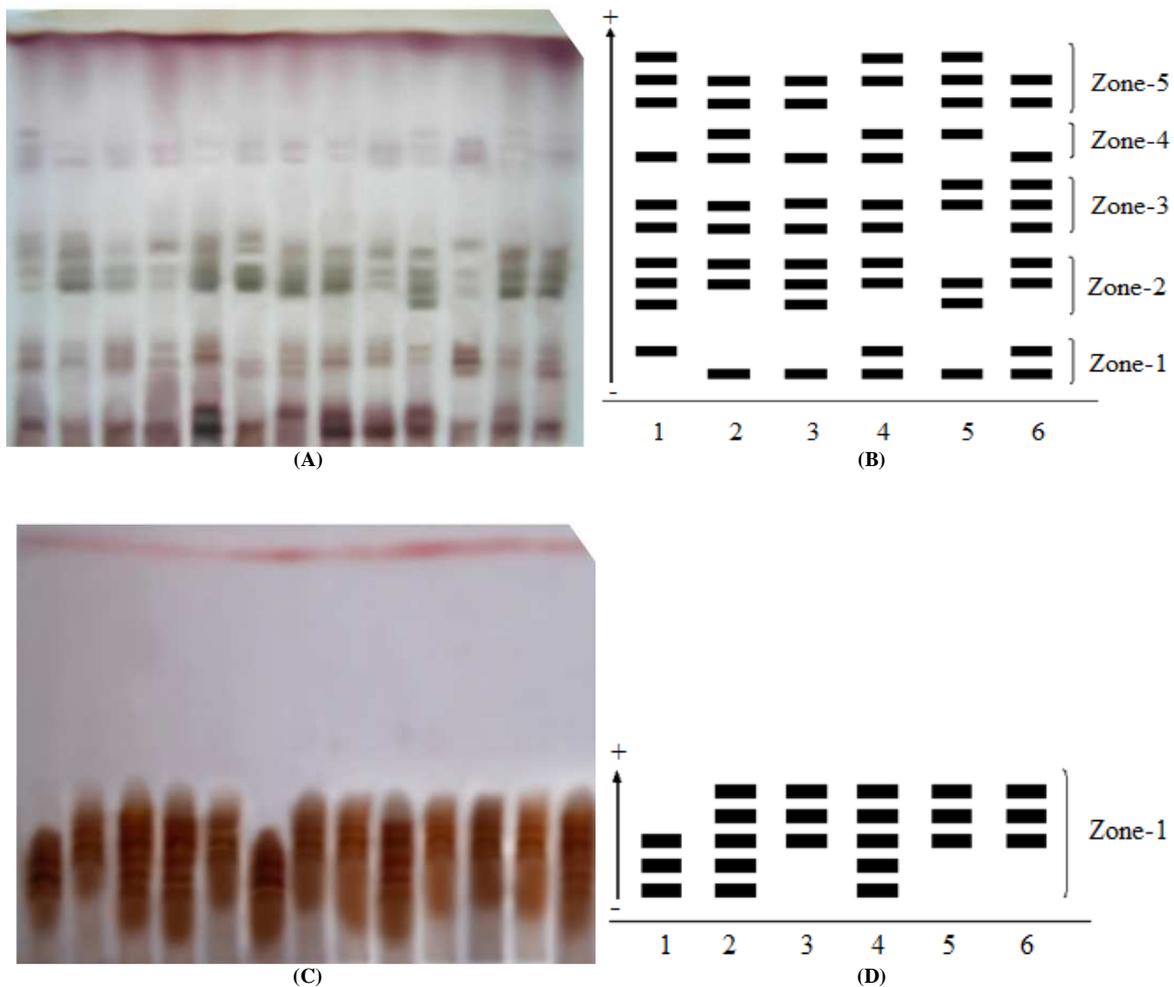


Fig. 1:

- (A) Photograph of gel showing banding pattern of Esterase in *S. oleoides*
- (B) Zymogram of Esterase showing banding pattern in *S. oleoides*
- (C) Photograph of gel showing banding pattern of Acid phosphatase in *S. oleoides*
- (D) Zymogram of Acid phosphatase showing banding pattern in *S. oleoides*

At locus-2 single banded variant corresponding to homozygous representing fast allele (F) and double banded variant corresponding to heterozygote (FS) had been observed, thus representing the monomeric nature of the enzyme [22]. However homozygotes corresponding to slow allele (S) were not found in the eleven populations analyzed. This might be due to the reason that the homozygous corresponding to slow (S) allele did not survive and thus eliminating this allele from the population.

**Peroxidase (PRX):** Five zones of activity were apparent on gels stained for peroxidase (Figure 2 (G) & (H)). The first two cathodal zones (Prx-1 and Prx-2) consisted of single banded pattern in all the eleven populations, showing no polymorphism, indicating monomorphic nature of enzyme at these loci. Locus-3 consisted of single and double-banded variants, which represented homozygotes (FF, SS) and heterozygotes (FS) genotypes respectively, thus establishing monomeric nature of the enzyme with two allelic variants [23]. Locus-4 was polymorphic with three co-dominant alleles (F, M and S) which exhibited single band for homozygous individuals and two bands for heterozygous individuals suggesting monomeric enzyme at this locus. The most anodal zone (Prx-5) did not show any polymorphism thus establishing monomorphic nature of enzyme.

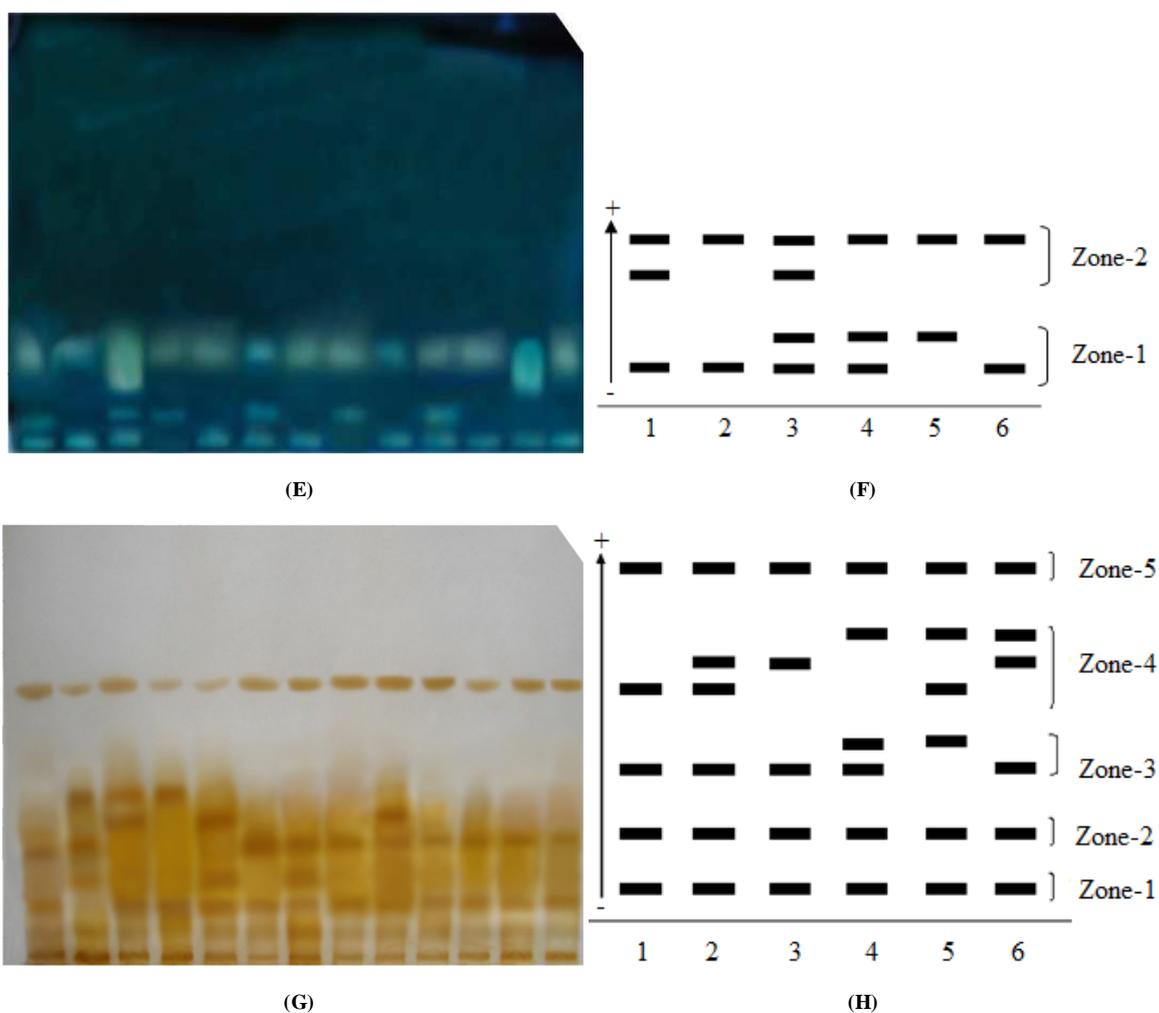


Fig. 2:

- (E) Photograph of gel showing banding pattern of Catalase in *S. oleoides*  
 (F) Zymogram of Catalase showing banding pattern in *S. oleoides*  
 (G) Photograph of gel showing banding pattern of Peroxidase in *S. oleoides*  
 (H) Zymogram of Peroxidase showing banding pattern in *S. oleoides*

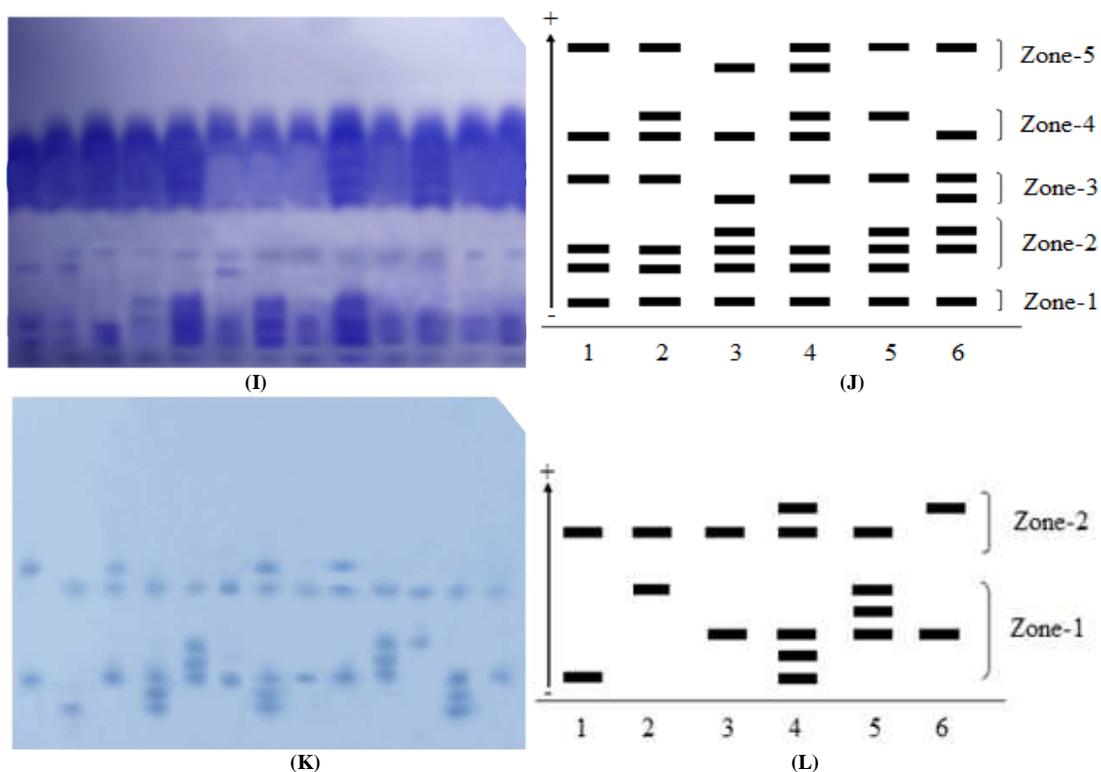
**Malate dehydrogenase (MDH):** Gels stained for MDH showed five zones of activity, which were considered as products of five loci (Mdh-1, Mdh-2, Mdh-3, Mdh-4 and Mdh-5) (Figure 3 (I) & (J)). At Mdh-1 locus no polymorphism was observed representing monomorphism at this locus. At Mdh-2 locus double banded and triple banded pattern was observed which correspond to homozygous (FF, SS) and heterozygous (FS) genotypes. The present observation in MDH could be explained on the basis that more than one electromorphs (conformational isozymes) may arise due to posttranslational differential binding of NAD coenzyme. Thus the subunit structure of enzyme was dimeric at this locus, which showed two banded and three-banded pattern due to posttranslational

modifications. The present observation in MDH concur with earlier report that in NAD requiring dehydrogenases more than one electromorphs (conformational isozymes) may arise due to posttranslational binding of coenzyme NAD [20, 24]. At locus-3 two alleles were identified which produced single banded enzyme at this locus. At loci Mdh-4 and Mdh-5 also single banded and double banded pattern was observed corresponding to monomeric isozymes at these two loci.

**Phosphoglucosomerae (PGI):** Two zones of activity were revealed on the gels stained for this enzyme representing two polymorphic loci Pgi-1 and Pgi-2 (Figure 3(K) & (L)). At locus one (Pgi-1) three alleles (F, M, S) were identified. Homozygous individuals were single banded (FF, MM, SS) and heterozygous individuals (MS and FS) were triple banded, demonstrating dimeric nature of Pgi-1 with two parental homodimers and an additional product of intermediate mobility. At locus two (Pgi-2) single bands for homozygous individuals and two bands for heterozygous individuals were obtained suggesting monomeric nature of isozyme at this locus.

Thus, the banding pattern of heterozygous genotypes revealed subunit structure of the enzyme i.e. occurrence of a hybrid band indicates dimeric structure while absence of hybrid band revealed monomeric enzyme [19]. Similar results have been obtained in a number of plants which demonstrated that PGI isozymes are dimeric [25-27].

**Phosphoglucumutase (PGM):** There were two polymorphic zones of activity on gels stained for Pgm (Figure 3(M) & (N)). Electrophoretic variants at the anodal staining zone segregated independently relative to those of the cathodal staining zone suggesting that two loci control PGM isozyme, Pgm-1 and Pgm-2. Three alleles (F, M and S) were identified at Pgm-1, which produced single banded homozygous and double-banded heterozygous genotypes. At Pgm-2 locus single banded homozygous (SS) and double-banded heterozygous genotypes (FS) were observed. Homozygotes corresponding to fast allele were not detected in the eleven populations analyzed. This might be due to the reason that homozygotes corresponding to fast allele (F) did not survive and thus eliminating this allele from the population. At both loci, heterozygous individuals showed double banded pattern thus indicating that *S. oleoides* PGM isozymes had a monomeric structure. Similar PGM zymograms banding patterns were widely reported [28-29].



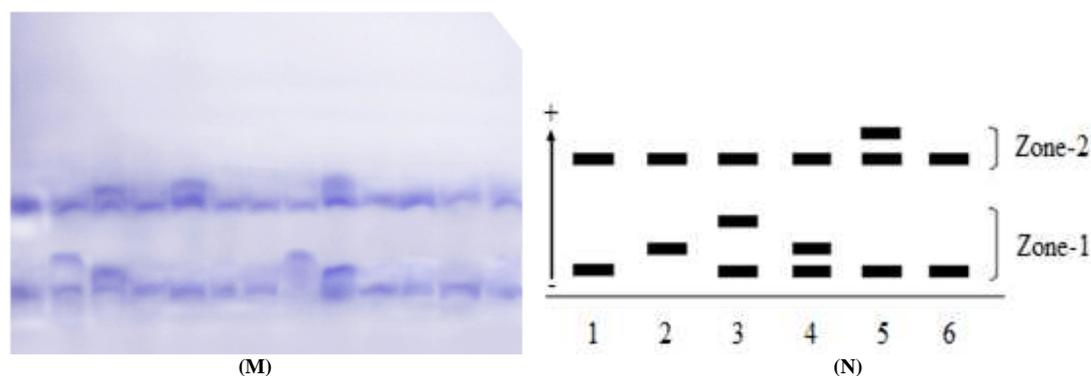


Fig. 3

- (I) Photograph of gel showing banding pattern of Malate dehydrogenase in *S. oleoides*  
 (J) Zymogram of Malate dehydrogenase showing banding pattern in *S. oleoides*  
 (K) Photograph of gel showing banding pattern of Phosphoglucosomerase in *S. oleoides*  
 (L) Zymogram of Phosphoglucosomerase showing banding pattern in *S. oleoides*  
 (M) Photograph of gel showing banding pattern of Phosphoglucumutase in *S. oleoides*  
 (N) Zymogram of Phosphoglucumutase showing banding pattern in *S. oleoides*

### CONCLUSION

In this paper emphasis was placed on native polyacrylamide electrophoresis of enzymes from simply prepared tissue homogenates, because these techniques are the most practical ones and are frequently applied in studies requiring scoring of several loci in large number of individuals.

Approximately 82% of the 22 enzyme loci in *S. oleoides* were determined as polymorphic. A total of 39 alleles were scored at these 22 loci.

These allelic data will help to estimate the genetic variation within and among *S. oleoides* populations.

### Acknowledgements

The authors are thankful to University Grants Commission, New Delhi for the financial assistance to carry out the research work under UGS SAP award.

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